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A COMPARISON OF THE RESULTS SECURED BY THE USE OF CHEMICAL AND PHYSIOLOGICAL ASSAY METHODS FOR TINCTURE OF ACONITE.

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Authorities are not in full accord as to the value of the official assay process for aconite and its preparations. Roth¹ states that this procedure gives no index of the activity of the drug. Anselmino² also condemns the use of the chemical method, and calls attention to the fact that the German Pharmacopœia no longer requires this useless performance. Gane and Webster,³ Lyons,⁴ and Robinson⁵ all present evidence to show that the determination of the so-called "aconitine" is no measure of the physiological activity of aconite preparations. Scoville,⁶ on the contrary, believes that the method is not devoid of value, in which stand he is supported by Stevens.⁷

Though the weight of recent evidence indicates that the chemical method for the assay of aconite is far from being strictly accurate, the fact that it is still required by the Pharmacopœia is our excuse for endeavoring to secure additional evidence as to the degree of concordance in the results obtained by the use of the chemical method and physiological methods.

For our experiments, four original pint bottles of tincture of aconite were secured through a local jobber, the object being to obtain samples from representative manufacturers. These samples were assayed by the process outlined in the U. S. P. VIII. The following results were obtained:

TABLE I.

Preparation	"Aconitine" Content
Tincture A04298
Tincture B04183
Tincture C04183
Tincture D04093

It has been urged that lethal-dose methods do not measure the therapeutic value of drugs. This objection may be valid in regard to the assay of such a drug as digitalis, which contains several important active principles that may, theoretically, decompose into substances that are highly toxic but possess no therapeutic value. With aconite, however, the matter is entirely different. Aconitine is by far the most important constituent,⁸ the other alkaloids which may be present contributing little or nothing to the physiological or toxic action of the aconite preparation. Under certain conditions, aconitine undergoes decomposition, but the resulting products are, relatively, devoid of toxicity. Therefore, since it seems safe to consider only one active principle, and since decomposition of this active principle leads to the formation of substances much less toxic and also much less active therapeutically, a lethal-dose method should give information regarding the amount of this substance present in the preparation to be assayed.

The determination of the lethal dose for guinea-pigs has been commonly accepted as the most trustworthy method for the physiological assay of aconite. In carrying out the tests according to this method, the tinctures were diluted in the proportion of one part of tincture to three parts of normal salt solution; the dose calculated per gramme body weight, and the injections made subcutaneously. The animals were examined at intervals over 24 hours, and note was made at the end of that time as to those that survived and those that succumbed. The method proved very satisfactory, in so far that the lethal dose can usually be determined with considerable accuracy, and it is not common for pigs to die much "out of order," though this occasionally occurs. The following protocol is illustrative of the results obtained:

EXPERIMENT I. *Tincture of Aconite A.*

Weight of Animal	Dose in Cc. per gram body weight	Results
465	.00025	Survived.
495	.00030	Survived.
235	.00030	Died.
240	.00030	Died.
260	.00032	Died.

The final results secured by the guinea-pig method are given in

TABLE II.

Preparation	M. L. D. in Cc. per gramme body weight
Tincture A00033
Tincture B00065
Tincture C00065
Tincture D00240

From this table, it is evident that the four tinctures vary markedly in their ability to cause the death of guinea-pigs. The extremes of toxicity are represented by Tincture A and Tincture D, the former being more than seven times more toxic than the latter. It will be remembered that, by the chemical method of assay, no great variations could be detected.

There are certain theoretical objections to the use of the guinea-pig method in assaying aconite preparations. It has been shown by Hunt⁹ and others that guinea-pigs are more susceptible to the action of certain poisons at one time of the year than at another; in other words, there is a seasonal variation in their resistance to the action of these poisons. The importance of this has been appreciated in regard to the use of the guinea-pig in assaying digitalis preparations, and we should not lose sight of the fact that a similar variation according to season may become evident when aconite is tested in this way. However, even should the animals show this seasonal variation in their resistance to the toxic action of aconite, this can have no appreciable influence on the results secured when all the experiments are carried out during a relatively short period of time, as was the case with ours.

It has also been shown that alcohol interferes with the absorption of digitalis administered subcutaneously to guinea-pigs, but this possible factor in the aconite assay has been avoided by using a constant dilution. It is conceivable, however, that certain tinctures may contain relatively large amounts of extractive matter or "colloid material," and that this may hinder the absorption of the aconitine when the tincture is injected subcutaneously. In order to determine whether this was really a factor of importance, the four samples were tested on cats, according to Hatcher's method. The following results were secured:

TABLE III.

Preparation	Lethal dose for cats in Cc. per gramme body weight
Tincture A00041
Tincture B00172
Tincture C00230
Tincture D00388

It is evident, from an examination of Tables II and III, that the physiological methods give roughly concordant results. It is

our belief that this concordance would be greater but for certain factors that were present in the performance of the cat method, and which are now the subject of investigation.

A comparison of the results secured by the use of the three methods is given in Table IV:

TABLE IV.

Preparation	M. L. D. Guinea-pigs	L. D. Cats	Chemical Assay
Tincture A00033	.00041	.04298
Tincture B00065	.00172	.04183
Tincture C00065	.00230	.04183
Tincture D00240	.00388	.04093

SUMMARY AND CONCLUSIONS.

1. Four samples of tincture of aconite secured from representative manufacturers showed little variation in composition as determined by the official method of assay.
2. By tests of these same samples upon guinea-pigs, great variations in toxicity were apparent.
3. The results secured by the guinea-pig test were confirmed by the use of Hatcher's method.
4. The assay process demanded by the U. S. P. for tincture of aconite is not only useless but actually harmful in giving a false sense of security.

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- ⁸ Cushny, 6th Edition.
- ⁹ Bull. 69, U. S. P. H. and M. H. Service Hyg. Lab.

A MODIFICATION OF ROSE'S METHOD FOR THE ESTIMATION OF PEPSIN.*

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In spite of all the work that has been done to isolate pepsin in a pure condition the results have been negative; for this reason it is as yet impossible to offer a method for its quantitative determination. As a matter of fact, while a number of so-called quantitative methods have been devised, the results obtained are only of value from a comparative standpoint. Such comparisons, however, are of decided importance and any means which will further their approach to a standard basis are worthy of consideration. It is with this view that a modification of Rose's method is here offered.

It is not necessary to review all of the many methods proposed for the estimation of pepsin. Suffice it to say that those of Mett,¹ Jacoby-Solms,² and Rose³ are most used. Each has certain advantages at once apparent to a worker, but the rapidity and ease of the latter method, with the modification here offered, would seem to warrant its use. The Mett method has been in use so long that it is not necessary to describe the procedure. That of Jacoby depends on the digestion of a 0.5 per cent. solution of ricin in 5 per cent. sodium chloride, at 37° C. for three hours, with varying amounts of a $\frac{1}{100}$ diluted gastric juice. Rose's method demands the digestion of a 0.25 per cent. solution of pea globulin in 10 per cent. sodium chloride, at 37° C. for one hour or at 50° to 52° C. for 15 minutes, with varying amounts of a previously neutralized gastric juice usually diluted five times.

Against the use of both the Mett and the Jacoby methods can be offered at once the consumption of time, not to mention other factors. It is not always possible to get ricin and this led us to use the Rose method. It does not seem justifiable, however, to neutralize the gastric contents as Rose does. He lays much stress upon this point and says the failure to neutralize is a serious objection to the Jacoby method. He thinks the final acidity in each tube should be the same. In so neutralizing the method is rendered unreliable. Evidence is

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offered in the present paper sustaining this proposition and a correction is made.

Rose's complete method follows:

0.25 gram globulin of the pea prepared as described . . . is dissolved in 100 Cc. of 10 per cent. sodium chloride (by warming slightly if necessary) and filtered. Portions of the clear filtrate of 1 Cc. each are introduced into a series of 11 small test tubes about 1 cm. in diameter. To each tube is added 1 Cc. of 0.6 per cent. hydrochloric acid, and about five minutes are allowed for the development of the turbidity. A measured volume of the stomach contents is then exactly neutralized to litmus paper with dilute alkali. If a precipitate of acid protein forms, this is filtered off, and the clear neutral solution is diluted a known number of times (usually five) with distilled water, allowance being made for the dilution of neutralization. A portion of the diluted juice is boiled, and amounts decreasing by 0.1 Cc. added to the tubes of turbid protein; to the first, 1.0 Cc.; to the second, 0.9 Cc.; to the third, 0.8 Cc.; and so on to the eleventh, to which none is added. The unboiled juice is then rapidly added in increasing amounts, as follows: To the first, none; to the second, 0.1 Cc.; to the third, 0.2 Cc.; to the fourth, 0.3 Cc.; to the fifth, 0.4 Cc.; to the sixth, 0.5 Cc.; to the seventh, 0.6 Cc.; to the eighth, 0.7 Cc.; to the ninth, 0.8 Cc.; to the tenth, 0.9 Cc.; and to the eleventh, 1.0 Cc. Each of the tubes thus has a total volume of 3.0 Cc.; and a total acidity of 0.2 per cent. of hydrochloric acid.

The measurements of the solutions may be easily and accurately made with a pipette of 1 Cc. capacity, graduated to 0.01 Cc. The tubes are well shaken and allowed to stand in a thermostat or water bath for 15 minutes at a temperature of 50° to 52° C. Exactly the same endpoint is obtained by keeping the tubes at a temperature of 35° to 36° C. for one hour. At the end of the digestion time that tube in the series is selected which contains the least amount of gastric juice and which exhibits no turbidity. The peptic activity is calculated on the basis of the amount of gastric juice in this tube. The enzyme content is expressed by the number of cubic centimeters of the 0.25 per cent. globulin that would be digested by 1 Cc. of the undiluted gastric juice under examination, if the activity were exerted for a period of one hour at 35° to 36° C., or for 15 minutes at 50° to 52° C. For example, if 0.5 Cc. of a gastric juice diluted five times clears up 1 cubic centimeter of the 0.25 per cent. globulin solution in 15 minutes at the given temperature, the activity of the solution would be expressed:

$$\text{Peptic activity} = (1 \div 0.5) \times 5 = 10.$$

For clinical purposes it suffices to use the scale of pepsin units here proposed, which gives figures about one-tenth of those expressed on the Jacoby-Solms scale.

Rose further states:

In the above method the conditions are constant in every trial, in respect to acidity, volume, protein content, and temperature.

This statement is true enough, but in attaining this point it is

necessary first to neutralize, and in so doing a grave error is made. The effect upon pepsin of alkalis, even in dilution, is a question about which there is no dissension. Even very dilute alkalis will inhibit, if not destroy, the action of pepsin. The following experiment brings out the point: A 0.1 per cent. solution of scale pepsin was made containing 77.0 Cc. N/14 hydrochloric acid. 1 Cc. of this solution was exactly neutralized with 0.77 Cc. N/14 sodium hydroxide and diluted to 10. Another 1 Cc. portion, without neutralization, was diluted to 10. Both were now subjected to digestion at 50° to 52° C. for 15 minutes, as follows:

Pepsin Solution.

	Neutralized tubes.*					Unneutralized tubes.†				
	1	2	3	4	5	1	2	3	4	5
0.25 per cent pea globulin .	1	1	1	1	1	1	1	1	1	1
0.6 per cent HCl	1	1	1	1	1	1	1	1	1	1
Distilled water	0.9	0.7	0.5	0.2	0.0	0.9	0.7	0.5	0.2	0.0
10 x diluted pepsin	0.1	0.3	0.5	0.8	1.0	0.1	0.3	0.5	0.8	1.0

* No perceptible digestion in any.

† Complete digestion in 3, 4, and 5. Pepsin = $(1 \div 0.5) \times 10 = 20$.

Controls: Boiled pepsin + globulin + acid = negative. Water + globulin + acid = negative.

In the above case it is evident that the greatest part of the pepsin has been destroyed or inhibited. All of the pepsin has not necessarily been destroyed, but evidently enough to prevent digestion within the limit of time and temperature. If more pepsin and less acid were present, then after neutralization one would expect to find some pepsin. It seems that an enzyme is not destroyed immediately, in the way an acid solution can be neutralized. In neutralization of weak acid solutions with alkali as dilute as N/14 sodium hydroxide, there is no question but that, for the moment, there is an alkaline reaction at the point of contact with the alkali. Since this is true, if we partially neutralize a weak acid solution containing pepsin, there ought to be a reduction of pepsin. About the effect of alkalis on pepsin there can be no question; hence the above assumption is supported by the following experiment: Gastric contents were removed from two normal individuals, after an Ewald test meal, and these determinations made:

	Total acidity.	Free acidity.	Rose exact.	Proposed method.	Jacoby exact.	Jacoby neutralized.	Neutralized one-half, then Rose.
R.	55	42	Trace.	41	100	Trace.	16
M.	60	45	Trace.	41	100	Trace.	16

Goodman,⁴ working with the Jacoby method, concluded from a limited number of cases that a uniform acidity was not necessary. Rose takes exception to this and demands for his method a final uniform acidity. He states:

It will be seen that the enzyme content is expressed by much larger numbers when the total acidity is 0.05 per cent. of hydrochloric acid than when it is 0.2 per cent. This is not due to the greater activity of the pepsin under such conditions, but to the decreased turbidity of the globulin solution, resulting from the decreased acidity.

In part I can agree with him, but I do not see that this justifies the neutralization of the stomach contents under examination. His statement is true if the final acidity is low, but if 1 Cc. of 0.6 per cent. hydrochloric acid be added, then the additional acidity, present by virtue of that contained in the gastric contents, can have no influence on the turbidity, and hence is not misleading as to enzymolysis. The following typical experiment illustrates the point:

	Tubes.												
	I	2	3	4	5	6	7	8	9	10	11	12	13
1 Cc. of per cent. HCl.	0.6	0.3	0.15	0.075	0.0375	0.0187	0.0093	0.0046	0.0023	0.0012	0.0006	0.0003	H ₂ O
1 Cc. of 0.25% pea globulin..	I	I	I	I	I	I	I	I	I	I	I	I	I
Final total acidity, per cent..	0.3	0.15	0.075	0.0375	0.0187	0.0093	0.0046	0.0023	0.0012	0.0006	0.0003	0.0001	0.0

These tubes were allowed to stand 15 minutes, and at the end of that time tubes 1, 2, 3, 4, and 5 showed the same maximum turbidity, so far as perceptible to the naked eye. Tubes 6, 7, and 8 showed no maximum and progressively less turbidity. Tubes 9 to 13 were all clear, not even showing an opalescence. The same result was obtained at the end of an hour. To another series, with the above result at the end of 15 minutes, was added 1 Cc. of 0.6 per cent. hydrochloric acid; after 10 minutes, all the tubes showed a maximum turbidity, with no perceptible difference between them. That is to say, if the final total acidity is only 0.2 per cent., or increased to even 0.4 per cent., there is no apparent difference in the turbidity of the tubes. This certainly shows, from the standpoint of turbidity, that it is unnecessary first to neutralize the gastric contents. Even were the total acidity of a gastric juice under examination 100 to 120, the

final total acidity of the tube containing the greatest amount of diluted gastric juice would never reach 0.4 per cent.

In a series of 24 observations (Table I) on normal and pellagrous individuals, there are only two instances in which a value has been obtained by following exactly Rose's method. Strict attention has been paid to neutralization. Titrations have first been made with alizarin red and phenolphthalein. The first tinge of brown with alizarin red reacted neutral to litmus. In other instances the contents have been titrated to the first perceptible tinge of pink with phenolphthalein, and then, for the Rose method, another sample was made neutral to litmus by stopping short of this point. In no case has the reaction been alkaline.

TABLE I.

Case.	Total acidity.	Free acidity.	Pepsin number.		Remarks.
			Proposed method.	Rose method.	
1	91	70	50	Trace.	Normal.
2	60	45	41	Trace.	Do.
3	44	21	31	Trace.	Do.
4	61	44	41	12	Do.
5	83	67	41	Trace.	Do.
6	84	69	41	Trace.	Do.
7	61	46	25	Trace.	Do.
8	55	42	41	Trace.	Do.
9	71	49	41	Trace.	Do.
10	89	70	40	Trace.	Do.
11	69	49	42	Trace.	Do.
12	51	26	42	Trace.	Do.
13	58	42	42	Trace.	Do.
14	71	53	40	Trace.	Do.
15	78	64	33	12	Pellagra.
16	25	12	33	Trace.	Do.
17	76	57	41	Trace.	Do.
18	57	38	40	Trace.	Do.
19	45	25	25	Trace.	Do.
20	84	64	31	Trace.	Do.
21	56	31	21	Trace.	Do.
22	21	0	Trace.	0	Do.
23	35	7	12.5	0	Do.
24	14	0	Trace.	0	Do.

No attempt is made in this paper to take up the question of gastric digestion in pellagra.

That neutralization in the Jacoby method will in a like manner destroy or inhibit some of the pepsin, and consequently influence the results appears from the following:

Unneutralized juice.		Neutralized juice.		Remarks.
Proposed method.	Jacoby method.*	Rose method.	Jacoby method.	
41	100	Trace.	Trace.+	Normal.
41	100	Trace.	Trace.+	Do.
41	100	Trace.	Trace.+	Do.
41	83	Trace.	Trace.+	Pellagra.

The following method has been adopted in this laboratory.* The gastric contents are strained through cheesecloth. Two Cc. are measured by means of an Ostwald pipette into a 25 Cc. stoppered volumetric cylinder, and diluted to the mark with distilled water. Into each of seven small test tubes (1×10 cm.) is measured, with an Ostwald pipette, 1 Cc. of a 0.25 per cent. filtered pea globulin in 10 per cent. sodium chloride solution. To each tube is added 1 Cc. of 0.6 per cent. hydrochloric acid, also by means of an Ostwald pipette. The tubes are allowed to stand about 5 minutes, until the maximum turbidity develops. To the first five, distilled water is added as follows: To the first, 0.9 Cc.; to the second, 0.8 Cc.; to the third, 0.7 Cc.; to the fourth, 0.6 Cc.; and to the fifth, 0.2 Cc.; to the sixth and seventh, none. Then there are rapidly added to each test tube the following amounts of the $1/12.5$ gastric juice; to the first, 0.1 Cc.; to the second, 0.2 Cc.; to the third, 0.3 Cc.; to the fourth, 0.5 Cc.; to the fifth, 0.8 Cc.; to the sixth, 1.0 Cc.; and to the seventh, 1.0 Cc. of the diluted juice boiled. These measurements can be accurately made with a 1 Cc. pipette graduated in 0.01 Cc. All tubes are then immersed for 15 minutes in a water bath at 50° to 52° C. At the end of this time, the tube is selected which is clear and contains the least amount of diluted gastric juice. Upon this basis, the peptic activity is calculated as the number of Cc. of 0.25 per cent. globulin digested by 1 Cc. of undiluted gastric juice. For example, if tube 2 containing 0.3 Cc. of a 12.5 times diluted juice be clear, then the result would be expressed:

$$\text{Peptic activity} = (1 \div 0.3) \times 12.5 = 41.2.$$

Ordinarily this scheme of seven tubes is used, though it is not a rule. If the free acidity be high, sometimes a dilution of $1/25$ is made. The number of tubes used will depend upon the accuracy desired.

* I take pleasure in here thanking Dr. R. C. Lewis, of the staff, for the preparation of pea globulin used in these experiments.

	Tubes.						
	1	2	3	4	5	6	7
0.25 per cent. pea globulin Cc.....	1	1	1	1	1	1	1
0.6 per cent. HCl Cc.....	1	1	1	1	1	1	1
Distilled water, Cc.	0.9	0.8	0.7	0.5	0.2	0.0	0.0
Diluted gastric juice.....	0.1	0.2	0.3	0.5	0.8	1.0	0.0
Boiled gastric juice.....	0	0	0	0	0	0	1.0
Final volume, Cc.....	3	3	3	3	3	3	3
Peptic activity.....	125	62.5	41	25	17	12.5	0

The gastric contents are never filtered, but strained through cheesecloth, as it is believed in this way less enzyme is absorbed. The dilution has been kept at ten times, or greater, and water substituted for boiled juice to make up the final volume. This has been done in line with Nierenstein and Schiff's⁷ procedure, in order to lessen the amount of disturbing factors, be they proteoses, peptones, or antiferments. At the same time it serves in keeping the final total acidity nearer 0.2 per cent. than if boiled unneutralized juice were added.

The pea globulin is made according to Rose's description from the ordinary garden pea, *Pisum sativum*:

The finely ground peas, freed as much as possible from the outer coating, are repeatedly extracted with large quantities of 10 per cent. sodium chloride solution, the extracts combined, strained through fine bolting cloth, and allowed to stand overnight in large cylinders to deposit insoluble matter. The supernatant fluid is siphoned off and saturated with ammonium sulphate. The precipitate of albumins and globulins is filtered off, suspended in a little water, and dialyzed in running water for three days, until the salt has been removed and the albumins have been dissolved. The globulins are filtered off and washed two or three times with water, to remove the last trace of albumins. To purify further, the precipitate is extracted with 10 per cent. sodium chloride solution and filtered until perfectly clear. The resulting solution is exactly neutralized to litmus paper, by the cautious addition of dilute sodium hydroxide, and again dialyzed in running water for three days to remove the salts completely. The precipitated globulins are then filtered off and dried on a water bath at 40° C. During the complete process of separation, the proteins should be preserved with a mixture of alcoholic thymol and toluene. The globulins so prepared dissolve, practically completely, in 10 per cent. sodium chloride solution, and after slight acidification with hydrochloric acid yield a turbid solution which does not settle out on standing.

I have found this solution of globulin to settle out slightly in the case of controls, though as a rule it remains in suspension and certainly is much better than the precipitated ricin, which will settle out and rise to the top of the tubes. If preserved, as Rose directs,

with toluene, it can be kept for a month or more. On the assumption that exact neutralization only inactivated the pepsin, and that it might be reactivated at once, though this is not possible according to Tichomirow,⁵ the following experiment was tried: Some gastric juice was neutralized exactly to litmus with N/14 sodium hydroxide and at once the previously determined acidity was attained by adding N/14 hydrochloric acid. This juice was then subjected to the determination and no quantitative amount of pepsin found, while with the unneutralized juice there was a peptic value of 40. The pepsin can be determined in 25 minutes from the time the gastric contents are received, provided there are on hand stock solutions of pea globulin and 0.6 per cent. hydrochloric acid. Using a burette to deliver the globulin solution, the 0.6 per cent. hydrochloric acid, and the water, and graduated pipettes for the diluted juice, six determinations can be completed easily in an hour and a half.

The present method is recommended because it is believed to be as accurate as any and less time consuming. That it will check itself is shown by the following analyses on the same normal individual on different days:

Total acidity.	Free acidity.	Pepsin number.	Contents removed after—
			<i>Minutes.</i>
61	46	25	30
46	21	31	45
61	44	41	60
55	42	41	60
71	49	41	60
89	70	40	60
83	67	41	75
84	69	41	90

The low value (25) found above I believe is due to the fact that only 30 minutes elapsed after the giving of the test meal, as Hawk⁶ and his collaborators have found that the height of acid and pepsin secretion is not reached in that time. The high value (50) found (see Table I) in a normal individual may be due to a hypersecretion of pepsin, called forth by the high acidity (90). On the following day the same case with a total acidity of 60 showed a peptic value of 41. The limit for normal individuals found so far extends between 25 and 50. The following table gives a comparison of the results obtained on the same contents, by the proposed method and by that of Jacoby:

Case.	Total acidity.	Free acidity.	Pepsin number.		Remarks.
			Proposed method.	Jacoby method.	
1	51	26	42	100-	Normal.
2	55	42	41	100-	Do.
3	58	42	42	100-	Do.
4	60	45	41	100-	Do.
5	69	49	42	100-	Do.
6	71	49	41	100-	Do.
7	71	53	40	100-	Do.
8	89	70	40	100-	Do.
9	56	31	21	60-80	Pellagra.
10	57	38	40	100-	Do.
11	69	53	33	100-	Do.
12	84	64	31	100-	Do.

By "100-" is meant that the solution is almost clear, only a trace of turbidity being left, the tube containing the next higher concentration of gastric content being clear; therefore it is more exact to say "100-." From this table it is seen that the values obtained by my method correspond to about four-tenths of those of Jacoby-Solms.

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SOME RELATIONS OF PLANTS TO DISTILLED WATER AND CERTAIN DILUTE TOXIC SOLUTIONS.*

By M. C. MERRILL.

I. INTRODUCTION.

In view of the extensive use of distilled water as a medium in which to grow control plants for comparative purposes in solution-culture work, there is well-grounded justification for the performance of considerable experimental work in order to determine more definitely the relations of plants to this medium. The subject is an

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important one, and it will require much experimentation for the ultimate solution of all phases of the problem involved. While the results herewith reported are only preliminary in their nature, the fact that they give positive indications along certain lines has been deemed sufficient warrant for their publication at this time. In addition to determining the growth relations of plants in this and other media, consideration has also been given to the effect produced by growing plants in this medium as determined by means of electrical conductivity measurements.

II. HISTORICAL ASPECTS OF THE SUBJECT.

The relation of plants to distilled water is a matter that has been under more or less serious consideration at different periods for a long time. Woodward (1699), who first employed the method of water culture in 1691-1692 in his interesting experiments, found that plants grew better in river water than in either rain water, spring water, or distilled water. The difference was of course due to the quantity of plant food contained in the medium, and this idea, coupled also with the character of the nutrients, has been the basis for a vast amount of physiological work since that time.

Coming down to more modern times, there has been a diversity of opinion among the investigators of the subject in regard to the reason why plants and animals thrive so much better in natural water or aqueous media than in distilled water. Considering the period from about 1860 on down to the present, the most important explanations offered may be summed up under the following three heads:

1. Lack of essential nutrients;
2. The presence of deleterious substances;
3. Extraction of salts, or nutrient materials, from the organism immersed in the distilled water.

Holding each of these views there has been a formidable array of scientists at different periods, each group contending strongly to establish the correctness of its viewpoint.

Among the earlier workers in the field may be mentioned Boehm ('75), Dehérain ('78), and others, who believed that the lack of essential nutrients in the distilled water was responsible for the resulting poor condition of the organism. Boehm, for example, believed that calcium played a fundamental rôle in the metabolism of the plant, and that in its absence certain processes, notably that of

starch formation, could not be carried on and that therefore deterioration resulted. He also believed that calcium was necessary for the transfer of the reserve materials from the cotyledons to the formative organs. Dehérain repeated Boehm's experiments and confirmed his results.

Owing to the fact that even distilled water, which had been unquestioningly regarded as pure, produced effects simulating toxicity, a great deal of attention has been given in the past to the chemical and other properties of water distilled from different kinds of apparatus and under various conditions. On the animal side, workers, among whom may be mentioned Kölliker ('56) and Nasse ('69), had early noticed the injurious effects on tissues when the same were placed in distilled water. Nasse, for example, found the deleterious effect of distilled water about equal to that of the following solutions: 2.5 per cent. NaCl, 3.3 per cent. NaBr, 3.7 per cent. Na₂SO₄, and 5.0 per cent. NaI.

Nägeli ('93), in his classical work published twelve years after his death, found that very minute amounts of toxic substances, notably copper, in solution produced injurious effects on organisms (*Spirogyra*), and to this phenomenon he applied the term "oligodynamik" action. This line of work was extended to include other substances and other organisms, and claimed the attention at different times of Aschoff ('90), Loew ('91), Locke ('95), Ringer ('97), Copeland and Kahlenberg ('99), Dehérain and Demoussy ('01), Lyon ('04), Bokorny ('05), Hoyt ('13), and others. It is of particular interest to note that Ringer in some of his earlier work ascribed the injury to the extraction from the organism of necessary nutrient materials; but after the publication of Locke's experiments ('95), which Ringer duplicated and confirmed, the latter concluded that the injury done in the particular case under consideration (*Tubifex*) was due to deleterious materials in the distilled water. He says: "Copper in even infinitesimal quantities will disintegrate tubifex whilst water free from copper or other heavy metals and without any salts such as calcium salts can sustain the life of tubifex."

In regard to the third idea pertaining to the effects of distilled water on organisms, early workers, both on the plant and animal side, found that salts were extracted from organisms placed in distilled water, even though their methods for determining the extraction were somewhat crude. Among the early investigators on the animal side may be mentioned Plateau ('83), Ringer and his

school ('83, '84, '85, '94, '94a, '94b, '97), Loeb ('03), and others. The writer has another paper ready for publication in which is given a historical treatment of the subject of excretions from roots and other plant parts, so the discussion of certain phases of the plant work is reserved for that publication.

Upon the perfection and the employment of conductivity apparatus by physical chemists, it soon began to be used also by the various workers in the fields of soil, plant, and animal investigations. In this connection distilled water came in for its share of consideration. The determination of the purity of water by ascertaining its electrical conductivity speedily came into vogue, and it should be said that as far as electrolytes are concerned it is a very accurate and excellent method and has deservedly come into more and more general use for this purpose in the fields of chemistry, physics, and biology.

Koeppé ('98) for instance, determined the electrical conductivity of water obtained from various sources and compared his results with those of other workers. He believed that distilled water has a deleterious effect which is partly due to a withdrawal of salts necessary to the organism and partly to a swelling of the tissues. He was supported in his views by Oldham ('09), while Winckler ('04), Kobert ('05), and others argued in favor of the harmlessness of distilled water, especially in medical practice. Peters ('04) used the electrolytic conductivity method in his work on *Stentor* and found that there was an exosmosis of electrolytes when the organism was placed in distilled water, and he therefore concluded that the injurious effects noted were due to an extraction of salts. True and Bartlett ('12, '15, '15a) considered, for certain salts, not only the excretion but also the absorption of electrolytes under balanced and unbalanced conditions of the medium.

In a recent paper in which a historical discussion of the subject is also given, True ('14) concludes that over and above any injurious effects caused by deleterious substances in the distilled water there is still a "residuum of harmful action due to no known type of impurity." Because this harmful action seems to be most marked in water of least conductivity True believes that the withdrawal of electrolytes from the root tissues best accounts for the deleterious action, but that this withdrawal is "not due to the aggregate difference in osmotic pressure between the cells of the roots and the external medium." He chose lupine seedlings for his work because Frank ('88)

had found them very sensitive to distilled water. Schulze ('91), however, after several years of experience with *Lupinus luteus*, claimed that distilled water produced no toxic effects upon those plants.

Both before and after the appearance of the recent contribution by True just referred to, I carried on the investigations reported in this paper, which, as previously stated, are but preliminary in their nature, but which have given indications leading to the conception of an idea differing somewhat from the majority of those above mentioned regarding the relation between plants and distilled water. This conception will be briefly mentioned here, while the evidence and a further discussion will be given later; it is that pure distilled water is not harmful or injurious *per se*, but that because of the static condition forced upon them as a consequence of the absence of plant food, the growing cells become disorganized and thus become easy prey to bacterial and fungous action. Excretion of electrolytes does occur but this should be considered merely as a concomitant condition, or resulting effect of the conditions under which the plants are placed, and should not be considered as a *cause* of degeneration unless the electrolytes themselves be toxic.

III. METHODS.

(Germination, Culture and Conductivity.)

Canada field peas (*Pisum sativum*) and horse beans (*Vicia faba*), the small variety, were the plants selected, as both were known to be well adapted for growth in solution cultures. Of the various methods of seed sterilization tried out, the one in which the seeds were treated with 1-600 formalin-water for 15 minutes after being soaked for 24 hours in running water gave best satisfaction.

For germinating the seeds a modification of the method used by Boussingault ('74), and also by various investigators in the Bureau of Soils, was employed. This consisted in the use of ordinary enameled-ware pans about 12 inches in diameter and 3 inches in height, filled with tap water and covered with 6 × 6-mesh galvanized iron "hardware cloth," on which the previously soaked and sterilized seeds were placed. The seeds were then covered with filter paper or paper towelling which was kept moist throughout the germination process or until the radicles reached the water below. The germination was carried on in the greenhouse. In the course of four

or five days a splendid lot of vigorous, uniform seedlings which have serviceably straight radicles about 2 inches long with no laterals yet formed is obtained by this method; such seedlings are well adapted, both by their character and their accommodation to an aqueous medium, for solution-culture work. At this stage the plumules have grown to about one-half inch in length, and the plants are now ready for transfer to the culture medium, an operation which is easily and quickly done. This method of germination recommends itself both by reason of its simplicity and ease of operation and the certainty of securing excellent results. In the transfer process from the germinating pan to the culture medium, the entire seedling was always immersed and carefully rinsed in once-distilled and again in twice-distilled water; by this means the roots became free of any adhering impurities.

As containers for the cultures, ordinary glass tumblers were used, the sides of which were covered with black paper to prevent algal growth and the top covered with perforated paraffin paper. (For a complete description and illustration of the method see the paper by McCool, '13.) Ten plants were grown in most cases in each tumbler; exceptions to that number will be noted in each case when the series are discussed in detail. Galvanized iron wire supports were used to hold the plants upright when the seedlings had attained sufficient size to require them.

In all cases doubly distilled water was used, the second distillation being carried out in the laboratory with KMnO_4 added to the once-distilled water to oxidize any organic matter that might be present. Conductivity tests of this water showed it to possess a specific conductivity of 2.064×10^{-6} . The nutrient solution used was that of Pfeffer, redistilled water being the solvent for the necessary salts. Each tumbler was filled to a convenient level with either the water or the full nutrient solution as the case might be, approximately 250 Cc. being required. To replace transpiration loss, doubly distilled water was added as needed.

In the early days of conductivity work on solutions, measurements could be made only by means of a continuous current. Because of the resulting polarization effects, however, the resistance of the solution increased to such an extent as to introduce serious errors into the results. But thanks to the classical work of Kohlrausch and others, the alternating current method was devised and perfected, whereby the determinations became practically independent of

polarization effects. A vast amount of work has since been done in the realm of physical chemistry on conductivity measurements, a review of which, however, is outside the scope of this paper. For a clear and concise discussion of this subject see Jones ('09), Walker ('10), or Findlay ('10).

(To be continued.)

CONCERNING TWO SWEET-TASTING DRUGS.*

By PROFESSOR R. KOBERT, Rostock, Germany.

Professor Kobert, in a lengthy paper, discusses the value of the general chemical formula ($C_N H_{2N-4} O_{10}$) applied by him to saponins as a means by which saponin-like substances may be distinguished from other plant principles without the aid of biological tests, and gives several illustrations to prove the correctness of his statement.

Research work on this class of drugs along biological lines, he says, has thus far, in every instance, verified the chemical identification, inasmuch as all the substances belonging within the range of this general formula have proved biologically active when tested. At present the whole series from $C_{15} H_{22} O_{10}$ to $C_{26} H_{44} O_{10}$ is complete, some members even being represented by several saponins. All in all, about forty different substances belong to this series, some of which appear to be anhydrides, while others again seem to be hydrates of the general saponin formula. Professor Kobert recognizes the fact that there are still other saponins belonging to other general formulæ; nevertheless, by far the greater majority thus far studied belong within the range of this general formula. Recent investigation has added two more plant principles, viz., convallarin and eupatorin, to this series.

Convallarin with the chemical formula $C_{34} H_{62} O_{11}$, if written $C_{34} H_{60} O_{10} + H_2 O$, properly belongs to this series. But, as this analysis had been made some sixty years ago, it seemed imperative to repeat it in order to avoid error. Accordingly a chemical as well as a biological examination was made of the drug. The chemical examination verified the correctness of the formula for convallarin,

* Translated by Sister Bertha Mueller, P.D., German Hospital, Philadelphia.

and revealed the fact that both the convallarin of the market and that as it exists in the plant have this composition. *Convallaria majalis* contains two principles—an acid and a neutral convallarin. Both possess in a high degree the typical saponin action on blood.

The other principle, eupatorin, with the chemical formula $C_{42}H_{68}O_{20} + H_2O$, if simplified and written $2(C_{21}H_{34}O_{10}) + H_2O$, also belongs to this series. Curiously enough, this formula is already shared by four other saponins—guaiac bark saponic acid, achras saponin, helleborein, and strophanthinic acid, the latter being yielded by the seeds of *Strophanthus gratus*. Consequently it seemed highly important to test this new glucoside (eupatorin) biologically in order to determine whether it belongs to the saponin group or not. Inasmuch as the genus *Eupatorium* belongs to the very large family Compositæ, only few plants of which are known to contain saponins, the investigation promised to be very interesting indeed.

Eupatorin is found abundantly in the leaves of *Eupatorium rebaudianum Bertonii*, a plant growing wild in the mountainous districts of Paraguay. Further investigation showed that *E. cannabinum*, *E. ageratoides* L., and *E. purpureum* L. all contain saponins. Eupatorin is an ash-free, neutral, and well-crystallizable glucoside. It is soluble in water and alcohol, froths strongly in water, dialyzes in aqueous solutions, and consists of an acid and a neutral saponin. The latter has a sweet taste, is soluble in alcohol, and is not precipitated by lead acetate.

Dietrich isolated still another and apparently closely allied principle from the drug *E. rebaudianum*, which he termed rebaudin. This principle is even sweeter than eupatorin, being about 180 times sweeter than sugar, while eupatorin is about 150 times sweeter. Eupatorin and rebaudin, as well as the sapogenins derived from them on hydrolysis, possess marked hæmolytic action on both animal and human blood. This proves that eupatorin as well as rebaudin is a true saponin, and the value of this general formula ($C_NH_{2N-8}O_{10}$) as a guide has once more been proved.

Another sweet-tasting drug to which Professor Kobert has given attention is glycyrrhiza. This drug contains no less than four sweet-tasting principles—cane sugar, grape sugar, mannit, and glycyrrhizin, the latter being present in the drug in considerable quantity. Inasmuch as glycyrrhizin, like other saponin-containing drugs, contains saccharic acid in chemical combination, it seemed

logical also to look for saponins in it. According to its chemical formula, $C_{44}H_{84}O_{10}$, however, it cannot and does not belong within the range of our general formula. Nevertheless, it possesses well-defined physical and physiological properties identical with saponins—frothing in water, capability of holding insoluble matter in suspension, power to retard CO_2 gas from escaping from carbonated beverages, etc., facilitating moistening of substances which ordinarily are given to repel water, stimulating of mucous membranes, and the dialyzing of its aqueous solution, etc.

Since, according to good authority, there is as yet no reliable chemical test known by which glycyrrhizin may be distinguished from saponins, biological testing, the means by which saponins are unmistakably distinguished from other plant principles, had to be resorted to. By this means glycyrrhizin revealed itself as not being a true saponin, since it is absolutely devoid of any hæmolytic action on blood. Some true saponins are not hæmolytically active until properly purified. This being known, careful purification of the glycyrrhizin was resorted to, but it proved as inactive after purification as before. This does not, however, mean that glycyrrhizin does not belong to the saponins at large. It no doubt belongs to the lower group, the biologically inactive saponins.

On treatment with silver nitrate, glycyrrhizin yields a product medicinally equal to the silver albuminates. On hydrolysis by means of diluted mineral acids it yields, like other saponins, a hæmolytically active sapogenin. Here the very interesting fact was discovered that if taken internally it undergoes the very same changes in the intestinal tract of the living organism by virtue of the action of the ferments. These findings are of extraordinary significance to the expert chemist who may be called upon to testify before the courts in cases of mysterious deaths; for on finding this hæmolytically active but harmless substance in the intestinal tract of persons having taken licorice mixtures in some form or other, and having died suddenly from an unknown cause, the chemist might be misled and attribute death to it, because its action on blood is the same as that of the poisonous saponins.

Glycyrrhiza bark differs from the wood, in that it contains a considerable amount of resins, collectively known as glycyrrhiza resins. It is these resins which cause the bark to taste so markedly different from the wood. According to Professor Tschirch, they are

divided into an ether-soluble and an ether-insoluble resin, the latter having a bitter, disagreeable taste. Both resins are soluble in 96 per cent. alcohol.

The following tabulation gives the per cent. of resins in unpeeled glycyrrhiza coming from various sources:

Source of Drug	Per cent. of ether-soluble resin	Per cent. of ether-insoluble resin	Per cent. of total resins
1. Russia	4.12	8.16	12.28
2. Syria	3.03	9.16	12.19
3. Anatolia	2.35	10.04	12.39
4. Arabia	1.75	10.56	12.31
5. Italy	2.82	9.76	12.58
6. Alicante (Spanish)	3.37	9.18	12.45
7. Cordova (Spanish)	2.96	8.97	11.93
8. Zaragossa (Spanish)	2.07	8.78	10.85
9. Sevilla (Spanish)	2.00	10.75	12.75
10. Toledo (Spanish)	2.26	10.11	12.37

These resins are insoluble in water, but on boiling the drug, the method resorted to in the making of extract of licorice, they are dissolved by virtue of the glycyrrhizin. Hot water rendered alkaline also dissolves them. If a solution of these resins be acidified, a black precipitate is thrown down, which on being tested biologically proves hæmolytically active.

The ether-soluble resin, after proper purification with acetic ether, proved chemically, as well as biologically, identical with glycyrrhetic acid—the substance resulting from the hydrolysis of glycyrrhizinic acid. The ether-insoluble resin may be considered an alcohol-soluble substance, closely allied to glycyrrhizin. It, like glycyrrhizin, is biologically inactive, but on hydrolysis yields a hæmolytically active sapogenin.

SUMMARY.

1. A really hæmolytically active saponin does not exist in glycyrrhiza. The only biologically active substance is a preformed prosapogenin, which resides in the bark of the drug.

2. Glycyrrhiza pharmacologically belongs to the group of saponin-containing plants. It is, however, the mildest in the group and consequently the least harmful expectorant of all of them. Its agreeable, pure sweet taste makes it a valuable corrective for disagreeable-tasting medicinal substances. Properly-purified gly-

cyrrhizin is exceedingly sweet, a dilution of 1 in 20,000 still having a lasting pure sweet taste. This property should make it a valuable substitute for saccharin as a sweetening agent.

3. To the pure food chemist it is of value to know that it is practically impossible to distinguish by biological tests the true saponins from preparations made from unpeeled glycyrrhiza. There is, however, a chemical test; namely, the aglykones of glycyrrhizin yield on zinc-dust distillation naphthol derivatives, while those of the true saponins appear to be cholesterin derivatives, giving color reactions similar to those of the cholesterins.

4. The fact that the different saponins differ physiologically from each other is of significance to authorities and legislatures. The position taken by the Austrian Government, that saponins must be excluded from all foodstuffs, has become untenable, since saponins have been found to exist in spinach, lettuce, caraway, etc. Present laws concerning saponins should be so amended as to require that in every case the name of the plant which yields the saponin entering into the foodstuffs should be printed on the label.

Since our knowledge concerning the individual saponins is steadily growing, it is possible that before long all non-poisonous saponins will definitely be separated from the poisonous saponins.

QUARTERLY REVIEW ON THE ADVANCES IN MATERIA MEDICA AND PHARMACY.

By JOHN K. THUM, PH. G., Pharmacist at the German Hospital,
Philadelphia, Pa.

The chemical industry in this country continues to be in a somewhat chaotic state. Immediately after the beginning of the war there was a veritable panic, with everybody "up in the air" and everything up in price. This was subsequently followed for a while—a very short while—by a period of comparative quietness and assurances that everything would work out all right. But at the present time the chemical market is causing everybody to wonder what will happen next. Some chemicals are unobtainable, of some there is a great shortage, and nearly all are soaring in price.

When one considers the fact that nearly all plant life contains potash in some form it is hard to realize that there should be a shortage of potassium salts in this country.

It hardly seems likely that a country of the resources like ours will continue to remain dependent on other countries for such a necessity of life, for such indeed is potash! This country possesses resources that are bound to be developed. Witness how the dependence on Russia for a tasteless, odorless, and colorless liquid petrolatum was overcome under the stimulus of necessity.

Government experts are quite insistent that the seaweed along the Pacific coast will prove to be a very prolific source of potash. It is also quite possible that a practical and workable process for obtaining potash from feldspar will be developed. This should prove an illimitable source of supply, as feldspar is abundant in this country.

E. Meyer, in 1857, recommended a process which was essentially the ignition of 10 parts of feldspar (aluminum and potassium silicate) with 14 to 18 parts of lime, boiling the mass with water under pressure and evaporating the solution, at the same time conducting the products of combustion over the liquid in order to convert the caustic potassa into carbonate and to precipitate the alumina which was in solution. It is quite within the bounds of possibility that some modification of this process would result in a product that would be commercially profitable.

The lack of phenol in this country and the scarcity of those synthetics of which it is a source are also matters of grave importance. The matter of dyestuffs has also been a subject of much discussion in both the scientific and lay journals, to say nothing of the daily papers. Judging from the tone of what has been said at meetings held in various sections of the country, and what has been printed in the press, the consensus of opinion seems to be that governmental coöperation will be necessary if the coal-tar industry, with its many side lines, is to be put on a firm business basis.

When the Sixty-fourth Congress meets on Monday, December 6, it will have many important subjects to consider, not the least among them being national legislation which will protect in every honorable way the manufacturing and business interests of the country, and at the same time protect and promote the welfare of the country as a whole.

It may be of interest to pharmacists to know that not only are some of the better class of newspapers showing a growing disinclination to advertise patent medicines, but they are also beginning to discuss the proprietary evil. The *New York Sun* reported an

address delivered by Professor Francis Carter Wood before the Alumni of the College of Physicians and Surgeons, and in its editorial columns commented quite freely thereon. The gist of the *Sun's* editorial is a warning to the public to shun the proprietary-prescribing physician.

It impresses in unmistakable language the possibilities of harm to the patient in the acceptance of prescriptions from medical men who are too indolent or unscientific to think out and write their own prescriptions; who know nothing of the composition of what they prescribe except what the proprietor chooses to tell them, and who certainly have not the time nor the knowledge to find out if the manufacturer is telling the truth. It goes on to say that we "trust the doctor because we know him, and that this trust is misplaced if he prescribes a drug the composition of which he does not know."

That the newspapers could be of considerable influence in correcting this evil goes without saying, but their interest must become more general. (Editorial, *New York Sun*, October 7, 1915, through *Journ. A. M. A.*)

THE ASSAY OF BALSAM OF PERU.—The authors of this paper state that for the assay of this balsam a number of tests have been proposed and are to be found in the various pharmacopœias and similar publications; that those depending on color reactions are usually unreliable and of limited value. Much stress is laid on the acid value, saponification value, cinnamein content and its saponification value; that the determination of these constants is generally required by the best of authorities, and yet it is obvious, they add, that such values are far from being absolutely characteristic; that they are insufficient to demonstrate the purity or authenticity of a sample is a fact unpleasantly brought to their attention by the appearance, in recent years, of imitation or "synthetic" balsams almost indistinguishable from the natural product.

In a thorough examination of balsam of Peru made by Thoms there was isolated, among other things, a new alcohol, peruvial. This compound, a light liquid with a characteristic odor, was subsequently found to be identical with nerolidol, the sesquiterpene alcohol isolated by Hesse and Zeitschel from the high-boiling fractions of oil of orange flowers.

The authors believe that peruvial, with its high iodine value and dextro-rotation, is the most characteristic constituent of the balsam, and they advise the following simple method for isolating it:

Peruvial Test.—Twenty grammes of balsam are saponified by heating one hour on a water-bath, with frequent shaking, in a litre flask, with 20 grammes of 25 per cent. potassium hydroxide. Steam is then passed through the mixture, and the distillate collected in 100 or 150 Cc. flasks with narrow, graduated necks. From natural balsams there is obtained in this manner, in 300 Cc. total distillate, from 0.7 to 0.9 Cc. of light oil. Imitation balsam gives only traces of heavy oil. (By Francis D. Dodge and Alfred E. Sherndal, *Journal A. Ph. A.*, October, 1915, p. 1222.)

QUIZZES, TESTS AND EXAMINATIONS.—Repeated arguments against final examinations as a means of judging the ability of a student have failed to convince the writer of this paper that the time is ripe for their abolishment. They are the best incentive a student has to stimulate his interest and make him strive to do his best. But that best should not be postponed until just a few weeks before final examination. There should be consistent daily application on the part of the student. "There is no short road to success." There never was and never will be! The author's experience in teaching has convinced him that the most practical method of making students study all during their college course is to put quiz work on the basis of frequent written tests. (By H. V. Arny, *Journal A. Ph. A.*, October, 1915, p. 1233.)

CAMPBOR OIL IN OIL OF TURPENTINE.—To detect light camphor oil in oil of turpentine and to distinguish it from pine oil it is suggested to test for safrol, which is a constituent of camphor oil: 100 mls of sample are distilled, and the last 5 mls of distillate are treated with an equal volume of strong H_2SO_4 , added drop by drop, and cooling after each addition. The liquid is then added to 20 mls of water and extracted with 10 mls of amyl alcohol. On treating the alcoholic layer with 5 mls of solution of potassium carbonate (20 per cent.), a green or bluish coloration, changing to red on the addition of sulphuric acid, is produced if safrol be present. (*Ann. Chim. Appl.*, 1915, 3, p. 372.)

JAPANESE CARBOLIC ACID.—It appears, from the following news item, that the interesting people of Japan are getting busy to manufacture the necessities from coal tar: "The experimental production by the Tokyo Gas Company of benzol, naphthalene, carbollic acid, and even aniline from coal tar has been attended with results that inspire confidence." This company expects to be able to put out,

and no doubt is putting out by this time, nearly 15,000 pounds of phenol per month. It has erected a large factory on the outskirts of Tokyo for this purpose. It is hoped by those interested in this venture that they will soon be able to increase the output of phenol to 190 short tons. How long must we in this country wait until the conditions for the manufacture of phenol are changed for the better?

SELECTIVE STAIN FOR POTATO STARCH.—It is stated that metachrom red G (Agfa) affords a selective stain for the isolation of potato starch grains in bread or mixed with other starches. Thirty per cent. alcohol is saturated with the dye at boiling-point, and the solution cooled, filtered, and diluted with 25 per cent. of water. A small quantity of the substance to be examined is suspended in a drop of water on a slide, dried, and then moistened with a drop of the stain. The slide is quickly washed with water and again dried. Potato starch and cell tissue are stained an intense golden-yellow, but cereal starches remain unaffected. The sample must be perfectly neutral in reaction to render the test successful. (*The Druggists' Circular*, October, 1915, p. 665.)

DIGOSID.—There has been placed on the market a preparation termed Digosid. The claims made for it are that it contains the active principles of digitalis, digitoxin, and digitalin, and is free from saponins and other by-products that cause disagreeable after-effects. It is a white, amorphous powder, easily soluble in chloroform, methyl alcohol, ethyl alcohol, and benzene, but difficultly soluble in ether, water, and petroleum-ether. (*The Druggists' Circular*, October, 1915, p. 665.)

ADALIN.—A. Nieuwenhuijse reports a case of what appeared to be veronal poisoning, but on finding an empty box near patient it was shown to be adalin. Another illustration of the readiness of short, catchy coined names to lead the public on to self-medication with dangerous hypnotics. (*Journal of A. M. A.*, October 30, 1915, p. 1594.)

IODINE.—Iodine in the form of the tincture is recommended as a substitute for potassium iodide in diseases like syphilis, rheumatism, gout, and obesity. Another reason why wood alcohol should not be used in making this preparation. (*Journal A. M. A.*, October 30, 1915, p. 1594.)

CAMPHOR.—It is reported that a second years' crop of camphor has been obtained from the only productive camphor plantation in the United States, that at Satsuma, Florida. The yield is said to be

satisfactory. It is said that smaller plantations in various parts of Florida will soon begin to show results. This is the result of a campaign begun twelve years ago by the government. As more and more land is to be used for the cultivation of the camphor laurel in this section of the country, it is probable that in ten years this country will be independent of foreign camphor producers. (*Journal A. M. A.*, October 30, 1915, p. 1555.)

BETANAPHTHYL SALICYLATE.—Betanaphtholis Salicylas—Betan.—The salicylic acid ester of betanaphthol. This drug has been accepted for inclusion in "New and Non-official Remedies" by the Council on Pharmacy and Chemistry of the American Medical Association. It is claimed that betanaphthyl salicylate undergoes no change in the stomach, but is decomposed when it reaches the intestines by the pancreatic juice and intestinal secretions. It is also believed to be of use as an antiseptic in the bladder. It also has the antirheumatic properties of salicylic acid. The dose is from 0.30 to 0.50 Gm. It is also found on the market as a proprietary preparation under the name "Betol." It is obtained by heating betanaphthyl-sodium and sodium salicylate with phosphorus oxychloride at from 120° to 130° C.

It is a white, shining, crystalline powder, colorless and tasteless, melting at 93.2° C. It is insoluble in cold or hot water or glycerin, difficultly soluble in cold alcohol or turpentine, easily soluble in boiling alcohol, in ether, in benzene, and in warm linseed oil. It should not give a violet color with ferric chloride test-solution. (*Journal of A. M. A.*, October 30, 1915, p. 1553.)

A DELICATE REACTION FOR APOMORPHINE.—One part of apomorphine in 500,000 can be detected with the following test: To 5 mls of the solution of apomorphine 5 drops of a saturated solution of HgCl_2 are added, and then 5 drops of a 10 per cent. solution of sodium acetate. The mixture is boiled for a few minutes. On cooling, 1 to 2 mls of amyl alcohol are added, which on shaking is colored blue if apomorphine be present. (*Ann. Chim. Anal.*, 1915, 3, p. 61.)

FORMALDEHYDE FUMIGATION.—A two-ounce saturated solution of alum mixed with eight ounces of solution of formaldehyde and the mixture poured over one pound of slaked lime is an efficient and economical method of room disinfection. This amount is sufficient to fumigate 1000 cubic feet of room space. (*Meyer Bros. Druggist*, August, 1915, p. 245.)

MAGNESIUM GLYCEROPHOSPHATE IN TETANUS.—Magnesium glycerophosphate is said to be very efficient in the treatment of tetanus; in fact, far superior to the sulphate. It acts immediately, and the severest spasms rapidly subside under it, while in the moderately severe cases 10 mils of a 25 per cent. solution every three or four hours keep the convulsions under control and the patient free from nearly all pain. Six or eight injections of the solution have been given for days at a time without the least harm to the patient. The pulse does not alter under this dose, and tests on animals have shown that the glycerophosphate causes only a slight drop in the blood-pressure, as against a considerable drop under magnesium sulphate. (*Berlin. klin. Wochen.*, June 28, 1915.)

OIL OF TURPENTINE AS A HÆMOSTATIC.—A severe case of bleeding after an operation on the elbow resisted all treatment until the wound was packed with gauze soaked in turpentine. The hemorrhage, which had previously been severe and long continued, at once ceased. The successful use of this oil has been proved on many other occasions. It is chiefly valuable in secondary hemorrhage. The oil is also antiseptic. Doubt is expressed as to the value of the oil as a hæmostatic when taken in the mouth. (*Lancet*, July 31, 1915, p. 226.)

GOVERNMENT SUBSIDIES FOR CHEMICAL MANUFACTURERS.—The Japanese Government has taken action with regard to the encouragement of the manufacture of coal-tar colors and synthetic chemical products used in medicine which is similar in some respects to the British Government scheme for establishing this industry on a large and permanent basis in Great Britain. A bill has been passed by the Japanese Parliament which provides for the granting of subsidies to companies engaged in the manufacture of dyestuffs and chemicals in Japan, provided that more than half of the capital in any such company is subscribed by Japanese subjects. The subsidies will be for a period of ten years, and the amount will be sufficient to enable the payment of a dividend of 8 per cent. per annum on their paid-up capital. (*The Pharmaceutical Journal and Pharmacist*, September 18, 1915.)

STRENGTH OF PICRIC ACID AS AN ANTISEPTIC.—Picric acid has been used very successfully for the sterilization of the skin before operation in strengths of 2 or 3 per cent. in alcohol. Investigation has shown that the Rideal-Walker carbolic acid coefficient of picric acid is exactly 6; a 0.165 per cent. solution of picric acid has the

same bactericidal powers towards 24 hours' broth culture of typhoid bacteria as has the standard 1 per cent. solution of carbolic acid. (*Lancet*, September 11, 1915, p. 604.)

THE COUNCIL ON PHARMACY AND CHEMISTRY of the A. M. A. has accepted histamine hydrochloride, the hydrochloride of the base beta-iminazolyethylamine (histamine). Histamine is closely related to histidine, from which it differs in that one molecule of carbon dioxide has been eliminated. Histamine hydrochloride has a powerful contractile action on certain muscular fibres and a strong vasoconstrictor action. The available evidence does not warrant a recommendation for its therapeutic use, but it is a valuable reagent for the standardization of pituitary and similar preparations. (*Jour. of A. M. A.*, October, 1915, p. 1367.)

MERCURIALIZED SERUM has also been accepted by the same body. It is a solution of mercuric chloride in normal horse serum, diluted with physiologic sodium chloride solution. It is claimed to be of value in the treatment of syphilis, particularly of the cerebrospinal type. It may be given intravenously or intraspinaly. (*Jour. of A. M. A.*, October, 1915, p. 1185.)

GALYL.—A substitute for salvarsan and neosalvarsan. It is claimed that this drug is a definite chemical composed of two molecules of arsenobenzol linked doubly with two phosphoric groups. It is a yellow powder, insoluble in water, and has an arsenic content of 35 per cent.; also contains phosphorus to the extent of 7 per cent. It dissolves readily in water in the presence of sodium carbonate; in fact, the drug is marketed with the required proportions of sodium carbonate present. It is best given intravenously. Given intramuscularly, it causes pain, and, like salvarsan, may lead to necrosis. (*Lancet*, through *New York Medical Journal*, October, 1915, p. 822.)

A NEW DISINFECTANT.—A. F. Stevenson, of the U. S. Public Health Service, in a report issued October 8, 1915, makes mention of a disinfecting fluid made by the saponification of pine oil and rosin, using NaOH as the alkali. The result is a dark reddish-brown liquid, clear, thick, and of an oily consistency. When added to water it makes a milky emulsion. It has a phenol coefficient of between 4 and 6. Unlike the cresol preparations of a similar nature, it has a rather pleasant odor. (*New York Medical Journal*, October, 1915, p. 862.)

DETECTION OF ARACHIS OIL IN OLIVE OIL.—Arachis oil, more generally known as peanut or ground-nut oil, is quite frequently used

to sophisticate olive oil. The following method for its detection is suggested: One mil of the suspected oil is heated with 5 mils of an 8 per cent. alcoholic caustic potash solution for 4 minutes in a flask provided with a condensing tube. After cooling to 25° , 1.5 mils of a mixture of one volume of glacial acetic acid and two volumes of water are added, followed by 50 mils of 70 per cent. alcohol. If the solution is turbid, it is carefully heated until perfectly limpid, is then cooled gradually, and the temperature at which it becomes turbid is noted. Pure olive oil becomes turbid at 13.5° ; that containing 5 per cent. arachis oil becomes turbid at 16.9° ; that containing 10 per cent. becomes turbid at 19.8° , and so on. The higher the percentage of arachis oil present, the higher the temperature at which the suspected oil becomes turbid. (*The Druggists' Circular*, October, 1915, p. 665.)

SOME ASH DETERMINATIONS ON DIGITALIS.—Some commercial digitalis, as well as some digitalis grown under their own supervision, was examined for ash residue. The results obtained seem to justify the writers in making the statement that a pharmacopœial requirement of 10 per cent. ash would exclude some specimens that would be authentic and of good quality. (By E. L. Newcomb and M. H. Haynes, *The Druggists' Circular*, November, 1915, p. 739.)

PROGRESS IN APPLIED SCIENCE.

NEW METHOD OF MAKING SULPHURIC ACID.

A new method of manufacturing sulphuric acid, for which advantages are claimed, is suggested in United States Department of Agriculture Bulletin No. 283, "The Production of Sulphuric Acid and a Proposed New Method of Manufacture." The essential difference of the method is that the gases employed are drawn downward through a spiral flue in place of being drawn through lead chambers or intermediate towers. It is asserted that the resistance of gases to the downward pull and the constant change in their course through the spiral tend to mix them very intimately. The fact that the gases constantly impinge on the walls of the spiral flue, which can be cooled either by air or water, makes it practicable to maintain the gases at a temperature most favorable for the efficient yield of sulphuric acid.

In laboratory tests in which the spiral was utilized, practically all

the sulphur dioxide was oxidized to sulphuric acid, only traces being lost through escape or in the system. The lead spiral, the author points out, however, is not intended to replace the Glover tower, nor to do away with the Gay-Lussac tower.

It is believed that, while the lead spiral will take considerable lead, the great reduction it will effect in the chamber space will make it possible to construct a plant with considerably less lead than is required in the ordinary chamber system.

The new type of plant requires no other device to accelerate the reactions, occupies much less ground space, and would not need as large buildings, and therefore should decrease the initial cost of construction. The method, however, has been tried only on a laboratory scale, and the bulletin refuses to predict just how efficient the commercial plant would be, but states that all indications are that this method offers promise of being economically successful.

ARSENATE OF CALCIUM A PROMISING NEW INSECTICIDE.

As a result of extensive tests during 1912, 1913, and 1914 with different insecticides, the entomologists of the United States Department of Agriculture have found that calcium arsenate, a new insecticide, gives very promising results in the control of certain insects that do damage by chewing on fruit trees. Among the chewing insects against which the arsenate of calcium proved effective, in laboratory and field tests conducted at Benton Harbor, Mich., are the codling moth, the fall webworm, the tent caterpillar, and the tussock moth. The details of the various experiments are published in Department Bulletin No. 278, "Miscellaneous Insecticide Investigations."

In these tests the effects of arsenate of calcium, both alone and combined with lime-sulphur solution, were tested in comparison with arsenate of lead alone and in combination with lime-sulphur. The arsenate of calcium, as was the arsenate of lead, was used at the rate of 2 pounds to each 50 gallons of water. In all the experiments the arsenate of calcium gave very satisfactory results in killing the larvæ without burning the foliage. In a number of cases its killing action was somewhat slower than, but compared favorably with, the arsenate of lead. Since it can be produced more cheaply than the lead arsenate, it would appear to have distinct value, although it has not been sufficiently tested to permit recommending it unre-

servedly for general use. Where arsenate of calcium was combined with lime-sulphur it was, as a rule, even more effective as a poisoning agent than when used alone, and did not lessen the value of the latter as a fungicide. When these compounds are combined, the amount of foliage consumed by the larvæ is less than where the arsenate of calcium is used alone. In tests in 1914 a commercial arsenate of calcium (paste), arsenic oxide 18.82 per cent., with lime-sulphur solution, gave very excellent control of the codling moth in comparison with arsenate of lead and with unsprayed plats. Where arsenate of calcium was used, 98.79 per cent. of the apples were sound; arsenate of lead showed 99.44 per cent. of sound apples; while in the unsprayed test plats only 58.71 per cent. of the apples were free from damage.

The following will be found to be a convenient way of making home-made arsenate of calcium:

Stone lime (90 per cent. CaO).....	55 pounds
Sodium arsenate, fused (dry powdered) 65% As_2O_5	100 pounds
Water	26 gallons

Place the stone lime in a wooden container and add a small amount of water, just enough to start slaking. When slaking is well under way, pour in the sodium arsenate, which should first have been dissolved in hot water. Keep stirring until the lime has thoroughly slaked. Sufficient water should be added from time to time to prevent burning.

The resulting arsenate of calcium should contain about 18 per cent. of arsenic oxide. In making this compound it will, of course, be necessary to know approximately the calcium oxide and arsenic oxide content of the materials employed and to vary the formula accordingly. The by-product is largely sodium hydroxide, which should be decanted if the insecticide is to be used on tender foliage.

EFFECTS OF SELECTION ON ALKALOIDS IN BELLADONNA.

Under the title "Some Effects on the Production of Alkaloids in Belladonna," the United States Department of Agriculture, in Bulletin 306, gives the results of a series of tests on controlling pollination of first- and second-generation plants, with especial attention to cross-pollination and close-pollination. Following are the conclusions reached in the bulletin:

It having been established in the previous investigation that a

wide range of variation exists in the alkaloidal content of belladonna plants, the present investigation was undertaken to determine whether the characteristic of alkaloid production is transmissible to the progeny through seed, and whether the character is changed by vegetative propagation. The results thus far show that the first-generation plants secured from seed of cross-pollinated selected individuals display the characteristic of the maternal parent with regard to alkaloid productivity. This condition is generally true at all stages of growth during a season, and also for at least two successive seasons. Close-pollination of the parent plant has shown only a moderate influence on the transmission of this characteristic.

Second-generation plants from cross-pollination have been grown at Arlington, Va., Madison, Wis., and Timmonsville, S. C., and at all three stations they have displayed the relative alkaloid-producing tendencies evident in the original parent plant and the generation preceding.

While the plants at the different localities showed a parallel relationship toward each other, there was considerable difference in the general quantity of alkaloids produced. Thus, in the case of Madison and Arlington, where two pickings were made at fairly corresponding stages of growth, it was found that the Madison plants yielded more alkaloids than those at Arlington. At Timmonsville the yield was still greater than at Madison, but here only one picking was made, and it is hardly possible to make a true comparison. Nothing definite developed to indicate that a relationship exists between the amount of precipitation and sunshine and the percentage of alkaloids produced.

Plants were grown from cuttings, and at two stages of their growth these plants showed a marked tendency to display the same characteristic regarding alkaloid production as the plants from which they were propagated and the original parents of those plants.

BOOK REVIEWS.

SCIENTIFIC AND APPLIED PHARMACOGNOSY. Intended for the use of students in pharmacy, as a hand-book for pharmacists and as a reference book for food and drug analysts and pharmacologists. By Henry Kraemer, Ph.B. (in Chemistry), Ph.M. (in Pharmacy), Ph.D. (in Botany); Professor of Botany and Pharmacognosy, and

Director of the Microscopical Laboratory, in the Philadelphia College of Pharmacy; Member of the Executive Committee of Revision of the Pharmacopœia of the United States of America; Corresponding member of the Société de Pharmacie de Paris, etc. Illustrated with over 300 plates, comprising about 1000 figures. Published by the author, 145 North Tenth Street, Philadelphia.

Drug analysts and others interested in the collection and commercial distribution of the vegetable drugs have long felt the need of an authoritative work on Pharmacognosy, particularly one which would embrace the largely-used non-official drugs. Many of the latter are in as great, or greater, demand than some of those recognized by the Pharmacopœia. The necessity for such a work as the book in hand will be apparent to all concerned.

In recent years greatly-increased interest in Pharmacognosy has been shown. The drug collector now finds it advisable to obtain the opinion of the trained pharmacognocist before collecting on an extensive scale. Similarly the drug merchant and the manufacturing pharmacist find it necessary to secure the opinion of the pharmacognocist before making purchases, and to see that incoming material is right.

It is essential that the student of Pharmacognosy should possess a working knowledge of chemistry and of botany, especially inner morphology. The latter subject is covered in an admirable manner in "Applied and Economic Botany," a companion book to the present one and a book with which the student should first familiarize himself.

It is obviously impossible, in the space that may be devoted to a review of this kind, to more than outline in a very general way a few of the many excellent features of the book before us.

This book of some 850 pages and 4000 titles embraces nearly all of the largely-used drugs and many of the rarer ones. The medicinal plants are grouped according to the Natural System of Classification of Engler and Prantl. The nomenclature adopted has been largely based on Engler and Prantl and the Index Kewensis, or, in the case of plants growing in the United States, on Gray's New Manual of Botany as revised by Robinson and Fernald.

The author divides the plants yielding medicinal products into three great groups, each of which is divided into two important sub-groups, as follows: Thallophytes, embracing Algæ and Fungi; Archegoniates, embracing Bryophytes and Pteridophytes; and Sper-

mophytes, embracing Gymnosperms and Angiosperms. The Angiosperms yield by far the greater portion of the vegetable drugs, and about seven-eighths of the book is taken up in consideration of the drugs of this group.

Special mention should be made of the number and excellence of the plates with which the book is illustrated. While the greater portion of the plates are reproductions of original photographs and drawings made by the author, in some instances the works of other authors have been drawn upon, credit being given to each author in connection with the reproduction. The selection of the borrowed illustrations has for the most part been a happy one, but in one instance a drawing by Hasse, representing a cross-section of *Helonias rhizome*, omits the most characteristic feature of the drug. Instances of this kind, however, are rare, the illustrations with their accompanying legends forming a most useful portion of the book, particularly to the student. In some cases the illustrations supplement the description given in the text, and the student is advised to consult them freely.

The individual drugs are treated in a most exhaustive and thorough manner, usually after the following outline: The Latin and English names, the habitat of the plant, the part used, the time of collection and method of preparation for market, a macroscopic description of the drug, the histology of the inner structure and the powder, the constituents, the allied products and adulterants, and, finally, literature citations.

A very useful feature of the book is the key for the study and identification of powders, in which they are first divided into groups according to color, sub-groups being formed by making use of the most important histological feature common to a portion of the larger group. A characteristic histological feature indicates the identity of the powder. By referring to the index the full description given in the text may be consulted.

A special feature of the book is the application of the crystallographic method of examination to certain drugs having constituents which may be obtained in crystalline form. In this method the angles and elasticity axes of crystals are determined by means of the petrographical microscope. The subject is in a rather rudimentary stage at present, but appears to offer an unlimited field for scientific investigation.

Mention should also be made of the unusually interesting and in-

structive chapters devoted to applied bacteriology, the poisonous fungi, and the animal drugs.

This comprehensive work, from the pen of America's foremost teacher of pharmacognosy and authority on histology, is the result of years of patient scientific investigation, and no doubt will receive the recognition it deserves.

JOHN MOSER, JR.

WINDOW DISPLAYS FOR DRUGGISTS. Comprising for the most part engravings and descriptions of over a hundred attractive displays which have been designed and used with success by druggists throughout the country, together with some useful suggestions on the subject of window dressing in general. Third edition, containing 32 new displays. Edited by Harry B. Mason, editor of the *Bulletin of Pharmacy*. Published by E. G. Swift, Detroit, Mich.

This neat, well-printed, and well-illustrated little volume of 190 pages is well worth the serious study of the retail pharmacist who wishes to learn how to make the most of his store and window space. People are very apt to judge the ability of a pharmacist to put up prescriptions and otherwise dispense medicines not only by his personal appearance, but also by the appearance of his place of business. Ability and merit are absolutely necessary if one wishes for success in one's business, but it is very materially helped along by a proper regard for the principles that govern the art of advertising. We know of no better way for a retail pharmacist to advertise than to take advantage of his room and window space to impress on his neighbors that he is a live one: a clean store and well-arranged windows to show his customers, and neatness and cleanliness to show those physicians who may come behind his prescription case.

This book can be purchased for the small sum of one dollar, and is worth every cent of it.

J. K. THUM.

BACTERIOLOGICAL METHODS IN FOOD AND DRUG LABORATORIES, WITH AN INTRODUCTION TO MICRO-ANALYTICAL METHODS. By Albert Schneider, M.D., Ph.D. (Columbia University), Professor of Pharmacognosy and Bacteriology in the College of Pharmacy of the University of California, San Francisco. P. Blakiston's Son & Co., 1012 Walnut Street, Philadelphia, Pa. Price, \$2.50 net.

Dr. Schneider is always interesting. He has written books deal-

ing with the general principles of microscopy, lichenology, powdered drugs, pharmaceutical bacteriology, and cultivation of medicinal plants. This new book strikes in a new field and will be much appreciated by his colleagues. It is primarily intended as a guide to students who are interested in the bacteriological examination of foods and drugs. Because of the close relationship between the work of the bacteriologist and micro-analyst, it is desirable that wherever possible these two laboratory branches be combined in the most effectual coöperative manner. The volume in hand is divided into two parts, the one giving an outline of micro-analytical methods in food and drug laboratories, and the second giving the bacteriological methods employed. The following subjects are considered: Grouping of Substances to be Examined in Food and Drug Laboratories; The Work of the Micro-analyst in Relationship to that of the Chemist and Bacteriologist; Equipment for Micro-analytical Work; Organoleptic Testing; Methods Useful in the Examination of Vegetable Drugs, Spices, etc.; Methods Useful in the Examination of Vegetable Products; Micro-chemical Color Reaction Tests; Making Analytical Reports; The More Important Histological Elements of Plants; Direct Bacteriological Examinations; Numerical Limits of Microörganisms in Foods and Drugs; Quantitative Estimations by the Cultural Methods; Preparation of Standard Culture Media; General Suggestions; Preparation of Required Standard Culture Media; Technic for Making Quantitative and Qualitative Estimations by the Plating Methods; Practical Application of the Quantitative Estimations by the Plating Methods; Qualitative Determinations; Evidence of Sewage Contamination; Possible Contamination of Foods with the Typhoid Bacillus; Possible Contamination of Food Substances with the Cholera Bacillus; Biological Water Analysis; Bacteriological Examination of Mineral Waters; The Microscopical and Bacteriological Examination of Milk; The Bacteriological Examination of Shellfish; The Bacteriological and Toxicological Examination of Meats and Meat Products; The Bacteriological Examination of Eggs and Egg Products; The Bacteriological Examination of Pharmaceutical Preparations; The Microscopical and Bacteriological Examination of Syrups; The Microscopical and Bacteriological Examination of Fermented Foods and Beverages; Standardization of Disinfectants; Determining the Purity and Quality of Sera, Bacterins, and Related Products; Special Biological and Toxicological Tests.

INDEX

TO VOLUME 87 OF THE AMERICAN JOURNAL OF PHARMACY.¹

AUTHORS.

Aguizy, Ahmed M. El. Ancient and Modern Pharmacy and Medicine in Egypt	500
Alpers, William C. Remarks on Digitalis.....	210
Bailey, E. Monroe. The Qualitative Separation and Identification of Some Oxymethylantraquinones	145
Beringer, George M. The Pharmacy and Materia Medica of the British Pharmacopœia of 1914	360
A Synopsis of H. R. No. 6282.....	131
Wine of Beef and Iron	406
Beringer, George M., Jr. A Comparison of the Value of Various Flavorings for Emulsion of Cod-liver Oil	115
A Device for the Rapid Sale of Nipples.....	377
A Medley	413
Blair, Henry C. The Pharmacy of Oxy-pinene.....	520
Blomén, J. Emile. Some Historical and Bibliographical Notes on the Therapeutics and Chemistry of the Oxy-pinenes	398
Some Studies on the Oxy-pinenes	199
Browne, C. A. The Development of the Sugar Industry.....	10
Chattopadhyay, Probodha C. The Examination of Chaulmoogra Oil...	473
Crosbie, H. H. The Cutting of Micro-sections.....	198
Dixon, Samuel G. A Substitute for Potassium Permanganate to Liberate Formaldehyde Gas from a Water Solution.....	62
Doolittle, R. E., and Burnett B. Wright. Some Effects of Storage on Coffee	524
Duggar, B. M. and M. C. Merrill. The Effect of Certain Conditions upon the Acidity of Tomato Fruits	277
England, Joseph W. Arsenic Antidote	59
An Historic Drug Store	259
The San Francisco Meeting of the American Pharmaceutical Association	483
Eve, A. S. Modern Views on the Constitution of the Atom.....	183
Farwell, Oliver A. Belladonna and Hyoscyamus.....	98
The Proper Time to Collect Sanguinaria.....	97
Ford, C. P. S., J. C. Ford, and J. E. Wine. A Comparison of the Guinea-pig and Cat Methods for the Physiological Standardization of Aconite Preparations	489
Ford, J. C., C. P. S. Ford, and J. E. Wine. A Comparison of the Guinea-pig and Cat Methods for the Physiological Standardization of Aconite Preparations	489
Givens, Maurice H. A Modification of Rose's Method for the Estimation of Pepsin	541
Green, Frederick R. Modern Medicine and Its Social Responsibilities	227, 280

¹ Compiled by M. G. Smith.

Hamilton, Herbert C. Disinfection. What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes..	309
Haskell, Charles C., and H. W. Zirkle. A Comparison of the Results Secured by the Use of Chemical and Physiological Assay Methods for Tincture of Aconite	537
Haynes, Hanley H., and Edwin L. Newcomb. Some Ash Determinations on Digitalis	112
Holmes, Ralph C., and Joseph L. Turner. Estimation of Cineol in Oil of Eucalyptus	101
Houseman, Percy A. Camphor Natural and Synthetic.....	49
Jacobs, Joseph. The Utilization of Our Own Resources in Producing Dyestuffs and Drugs	300
Johns, Carl O., and Arno Viehöver. On the Determination of Small Quantities of Hydrocyanic Acid	261
Kilmer, Frederick B. The Cultivation of Medicinal Plants.....	343, 421
Kobert, R. Concerning Two Sweet-Tasting Drugs	555
LaWall, Charles H. The Chemistry of the British Pharmacopeia 1914..	368
What is Rice Powder?.....	293
Le Boutillier, Theodore. Dextrinized Malt in Infant Feeding.....	162
Mayer, Joseph L. Inversion of Cane Sugar in Syrups.....	410
Standardization of Sodium Thiosulphate Volumetric Solution.....	154
McAnulty, John F., Jr. Some Reasons Why a Pharmacist Should Make His Own Preparations	117
McCord, Carey Pratt. The Pineal Gland in Relation to Somatic, Sexual and Mental Development	119
Merrill, M. C. Some Relations of Plants to Distilled Water and Certain Dilute Toxic Solutions	549
Merrill, M. C., and B. M. Duggar. The Effect of Certain Conditions upon the Acidity of Tomato Fruits	277
Miller, Reginald. The Rapid Determination of Small Quantities of Heroin	248
Motter, Murray Galt. Hookworm Disease. The Use of Oil of Chenopodium in Its Treatment	35
Mueller, S. Bertha. A Note on Compressed Tablets.....	197
Translation of "Concerning Two Sweet-Tasting Drugs," by Professor R. Kobert, Rostock, Germany	555
Newcomb, Edwin L. Ash Content of Some Unofficial Drugs.....	113
Belladonna and Hyoscyamus	I
and Manley H. Haynes. Some Ash Determination on Digitalis....	112
Peacock, B. L. DeG., and J. C. Peacock. Some Experiences with Salol-coating of Pills	514
J. C., and B. L. DeG. Peacock. Some Experiences with Salol-coating of Pills	514
Power, Frederick B. The Botanical Source, Physical Characters, and Chemical Constituents of Chaulmoogra Oil	493
Prescott, Samuel C. The Function of Enzymes	63
Rogers, T. B. Relation Between Druggist and Veterinarian.....	250
Rolfe, George W. Commercial Glucose and Its Uses.....	269
Romijn, G. The Estimation of Free Acid and of Oxychloride Solution..	245
Rosenthal, Helman, and P. F. Trowbridge. The Estimation of Fat.....	309
Sadtler, Samuel P. Some American Contributions to Industrial Chemistry	465

309	Sherndal, Alfred E. On the Blue Hydrocarbon Occurring in Some Essential Oils	157
	Shull, George H. A Pilgrimage to Brunn	69
537	Stiles, Percy G. Salt and Its Relation to Nutrition	220
112	Strong, W. W. Oxidation of Nitrogen, and How Cheap Nitrates Would Revolutionize our Economic Life	29
101	Thum, John K. Quarterly Review on the Advances in Materia Medica and Pharmacy	559
49	So-called Concentrated Solution of Silver Iodide	500
	Toplis, William G. Stearic Acid as a Coating for Enteric Pills	518
300	Trowbridge, P. F., and Helman Rosenthal. The Estimation of Fat	309
261	Turner, Joseph L., and Ralph C. Holmes. Estimation of Cineol in Oil of Eucalyptus	101
421	Viehöver, Arno, and Carl O. Johns. On the Determination of Small Quantities of Hydrocyanic Acid	261, 328
555	Warren, L. E. An Examination of Several Commercial Specimens of Opium Alkaloids or their Salts	439
368	Waters, Bertram H. An Experimental Study of a New Remedial Agent, and of Its Effects in Pulmonary Tuberculosis	163
293	White, J. Stanley. Bacterial <i>Versus</i> Vegetable Toxins	127
162	Wilbert, M. I. Drug Intoxication. An Economic Waste and a Menace to Public Health	136
410	The Number and Kind of Drug Addicts	415
154	The Pharmacy of Useful Drugs	373
117	Wine, J. E., J. C. Ford, and C. P. S. Ford. A Comparison of the Guinea-pig and Cat Methods for the Physiological Standardization of Aconite Preparations	489
119	Wright, Burnett B., and R. E. Doolittle. Some Effects of Storage on Coffee	524
549	Youngken, Heber W. The Comparative Morphology, Taxonomy, and Distribution of the Myricaceæ of the United States	391
277	Zirkle, H. W., and Charles C. Haskell. A Comparison of the Results Secured by the Use of Chemical and Physiological Assay Methods for Tincture of Aconite	537

SUBJECTS.

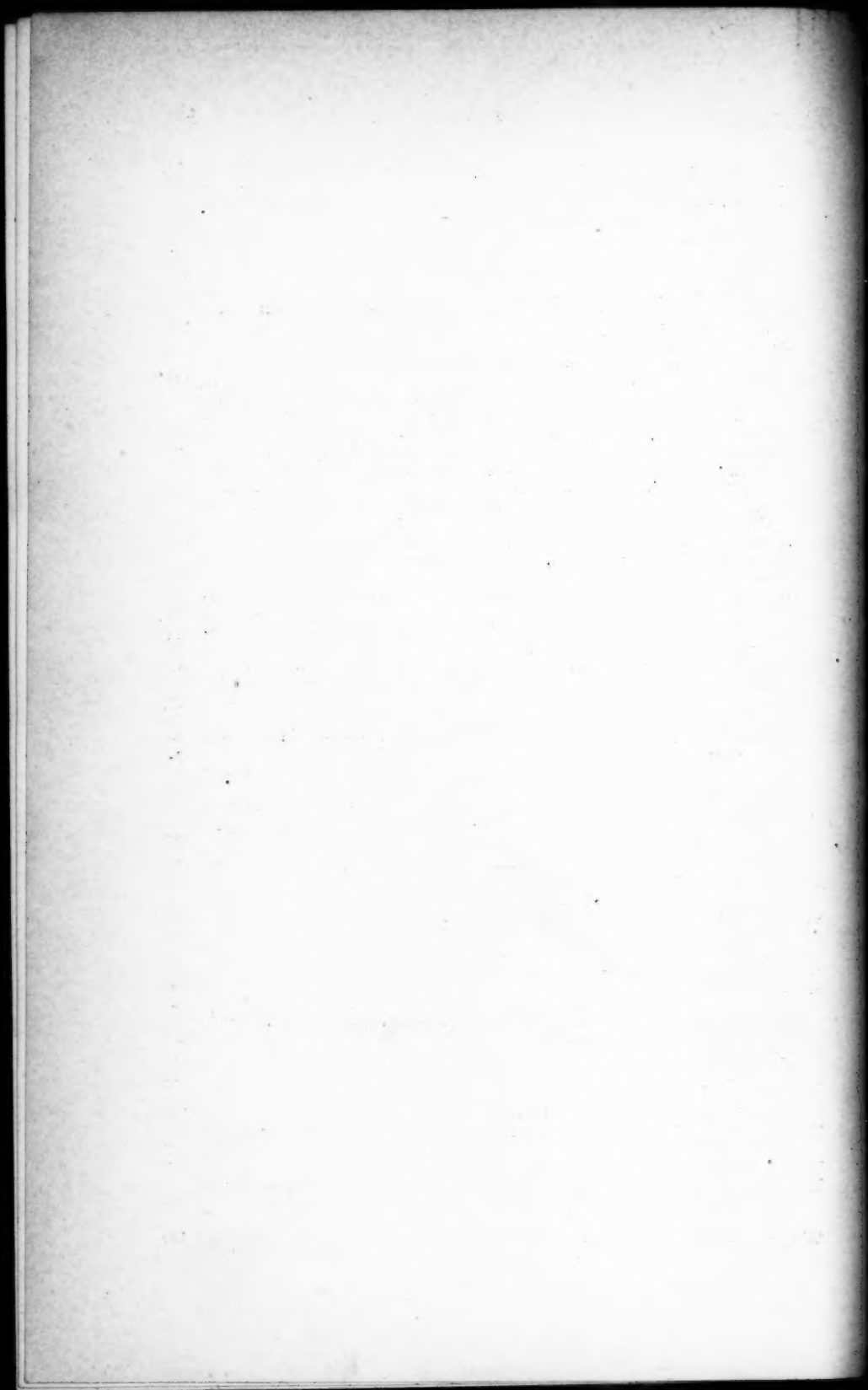
555	Acid, Carbolic, Japanese	562
113	Ester of Betanaphthol. The Salicylic	564
1	Free, and Oxychloride Solution, The Estimation of (Romijn)	245
112	Hydrocyanic, On the Determination of Small Quantities of (Viehöver and Johns)	261, 328
514	Hypochlorous, Antiseptic Action of, and Its Application to Wound Treatment	435
	Picric, Strength of, as an Antiseptic	565
514	Aconite Preparation, A Comparison of the Guinea-pig and Cat Methods for the Physiological Standardization of (Ford and Wine)	489
493	Tincture of, A Comparison of the Result Secured by the Use of Chemical and Physiological Assay Methods for (Haskell and Zirkle)	537
63	Adalin	563
250	Advanced Instruction in Pharmacy (Remington)	326
269	Agfa	563
245	Agricultural Alcohol	292
309	Algal Stalactites in Bermuda	40
	Alkaloidal Content of Stramonium Leaves	196
465	Antiseptic Action of Hypochlorous Acid and Its Application to Wound Treatment	435

Apomorphine, A Delicate Reaction for	504
Arsenic Antidote (England)	59
Ash Determination on Digitalis, Some	567
Atom, Modern Views on the Constitution of the (Eve)	183
Balsam of Peru, The Assay of	561
Beef and Iron, Wine of (Beringer)	406
Belladonna and Hyoscyamus (Farwell)	98
Belladonna and Hyoscyamus (Newcomb)	1
Beta-naphthol Reaction, New	143
The Salicylic Acid Ester of	564
Brunn, A Pilgrimage to (Shull)	69
Camphor	563
Natural and Synthetic (Houseman)	49
Oil in Oil of Turpentine	562
Cane Sugar in Syrups, Inversion of (Mayer)	410
Chaulmoogra Oil, The Botanical Source, Physical Characters, and Chem- ical Constituents of (Power)	493
The Examination of (Chattopadhyay)	473
Chemistry, Some American Contributions to Industrial (Sadtler).....	465
Chemical Constitution and Physiological Action	536
Cineol in Oil of Eucalyptus, Estimation of (Turner and Holmes).....	101
Coal-tar Dvestuff Industry	530
Cod-liver Oil, Emulsion of, A Comparison of the Value of Various Flavorings for (Beringer, Jr.)	115
Coffee, Some Effects on the Storage (Doolittle and Wright)	524
Cultivation of Medicinal Plants (Kilmer)	343, 421, 527
Determination of Small Quantities of Hydrocyanic Acid (Viehæver and Johns)	261, 328
Device for the Rapid Sale of Nipples, A (Beringer, Jr.)	377
Digitalis, Remarks on (Alpers)	210
Some Ash Determinations on	567
Some Ash Determination on (Newcomb and Haynes)	112
Digosid	563
Disinfectant, A New	566
Disinfection. What Disinfectant is the Most Generally Applicable for Clinical, Surgical, and Sanitary Purposes? (Hamilton).....	315
Drug Addicts, The Number and Kind of (Wilbert)	415
Intoxication. An Economic Waste and a Menace to Public Health (Wilbert)	136
Plants not Likely to Succeed, Inexperienced Growers of.....	42
Not Usually Profitable	328
Store, An Historic (England)	259
Drugs, Sweet-tasting, Concerning Two (Kobert) (Translated by S. Bertha Mueller)	555
The Pharmacy of Useful (Wilbert)	373
and Dyestuffs, The Utilization of Our Own Resources in Producing (Jacobs)	300
Unofficial, Ash Content of Some (Newcomb)	113
Druggist and Veterinarian, Relation Between (Rogers)	250
as Seen by the Governor of Minnesota, The	141
Dyestuff Industry, Coal-tar	530
and Drugs, The Utilization of Our Own Resources in Producing (Jacobs)	300
Enzymes Digestive	143
The Function of: Products of Living Cells that Affect the Chemical	

Operations of Living Matter but do not Become a Part of the Final Reaction (Prescott)	63
Eucalyptus, Estimation of Cineol in Oil of (Turner and Holmes)	101
Fat , The Estimation of (Trowbridge and Rosenthal)	309
Formaldehyde Fumigation	564
Substitute for Potassium Permanganate to Liberate the Gas from a Water Solution (Dixon)	62
Friedmann Treatment for Tuberculosis	76
Galyl	566
Glucose and Its Uses, Commercial (Rolfe)	269
Good Soil for Colon Bacilli	44
Government Subsidies for Chemical Manufacturers	565
Harrison Bill, A Synopsis of H. R. No. 6282 (Beringer)	131
Heroin, The Rapid Determination of Small Quantities of (Miller)	248
Hookworm Disease. The Use of Oil of Chenopodium in Its Treatment (Motter)	35
Hydrocarbon, Blue, On the, Occurring in Some Essential Oils (Sherndal)	157
Hyoscyamus and Belladonna (Farwell)	98
and Belladonna (Newcomb)	1
Iodine	563
Magnesium Glycerophosphate in Tetanus	565
Malt in Infant-feeding, Dextrinized (LeBoutillier)	162
Materia Medica and Pharmacy, Quarterly Review on the Advances in (Thum)	559
Medicinal Plants, The Cultivation of (Kilmer)	343, 421
Medicine, Modern, and Its Social Responsibilities (Green)	227, 280
Medley, A (Beringer, Jr.)	413
Micro-sections, The Cutting of (Crosbie)	198
Myricaceæ of the Eastern United States, The Comparative Morphology, Taxonomy, and Distribution of the (Youngken)	391
National Association of Boards of Pharmacy	336
Nipples, A Device for the Rapid Sale of (Beringer, Jr.)	377
Nitrogen, Oxidation of, and How Cheap Nitrates Would Revolutionize Our Economic Life (Strong)	29
OBITUARIES:	
Hance, Edward H.	88
Lee, William E.	91
Plaut, Albert, An Appreciation	380
Oil , Arachis in Olive Oil, Detection of	566
Camphor in Oil of Turpentine	562
Chaulmoogra,	473, 493
Chenopodium, in Hookworm Disease (Motter)	35
Eucalyptus, Estimation of Cineol in (Turner and Holmes)	101
Eupatorium	196
Olive, Detection of Arachis Oil in	566
of Turpentine as a Hæmostatic	565
Camphor Oil in	562
Wisconsin Wormwood	196
Oils, Essential, On the Blue Hydrocarbon Occurring in Some (Sherndal)	157
Opium Alkaloids or Their Salts, An Examination of Several Commercial Specimens of (Warren)	439
Oxymethylanthraquinones, The Qualitative Separation and Identification of Some (Bailey)	145
Oxy-pinenes, Some Historical and Bibliographical Notes on the Therapeutics and Chemistry of the (Blomén)	398
Some Studies on the (Blomén)	199
The Pharmacy of (Blair)	520

Pepsin, Estimation of, A Modification of Rose's Method for the (Givens) .	541
Peruvial Test	562
Pharmacist Should Make His Own Preparations, Some Reasons Why a (McAnulty)	117
Pharmacists, Belgian, In Aid of	48, 78
Pharmaceutical Association, American, The San Francisco Meeting of the (England)	483
Pennsylvania: Note of Annual Meeting, 1915.....	378
Pharmacopœia, 1914, The British.....	45
The Chemistry of (LaWall)	368
The Pharmacy and Materia Medica of (Beringer).....	360
of the United States of America, Digest of Comments of the.....	530
Pharmacy and Chemistry of the A. M. A., The Council on.....	566
and Materia Medica Quarterly Review on the Advances in (Thum) ..	559
Medicine in Egypt, Ancient and Modern (Aguizy)	501
PHILADELPHIA COLLEGE OF PHARMACY:	
Address of President French	238
Advanced Instruction in Pharmacy (Remington)	326
Annual Meeting	235
Minutes of the Board of Trustees.....	94, 243, 387, 535
Quarterly Meeting	93, 384
Semi-annual Meeting	533
Ninety-fourth Annual Commencement	330
Pharmaceutical Meeting	144
Pineal Gland in Relation to Somatic, Sexual and Mental Development (McCord)	119
Plants, Some Relations of, to Distilled Water and Certain Dilute Toxic Solutions (Merrill)	549
Potassium Permanganate, A Substitute for, to Liberate Formaldehyde Gas from a Water Solution (Dixon)	62
Potato Starch, Selective Stain for	563
Proceedings of the Eleventh International Congress of Pharmacy, held at The Hague, Holland, September 17 to 21, 1913.....	83
Quizzes, Tests and Examinations	562
REVIEWS, BOOK:	
Anderson, J. F., and Arthur M. Stimson. The Friedmann Treatment for Tuberculosis	88
Annals of the Missouri Botanical Garden, Anniversary Proceedings, St. Louis, Mo. (1915)	325
Annual Report of the Investigations of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the Amer- ican Medical Association	291
Askinson, George William: Perfumes and Cosmetics, Their Prepara- tion and Manufacture	326
Autenrieth, Wilhelm. Laboratory Manual for the Detection of Poisons and Powerful Drugs	224
Bessey, Charles E., and Ernst A. Essentials of Botany.....	37
Bliss, A. R. A Laboratory Manual of Qualitative Chemical Analysis for Students of Medicine, Dentistry, and Pharmacy.....	39
Churchill, J. and A. Squire's Pocket Companion to the British Phar- macopœia, 1914, Second Edition.....	225
Digest of Comments on the Pharmacopœia of the United States of America	86, 530
Dorland, A. Newman. Dorland's American Pocket Medical Dic- tionary	438
Hatcher, Robert A. The Pharmacology of Useful Drugs.....	529
Heckel, Edouard. Annales du Musée Colonial de Marseille fondées en 1893	324
Houghton, E. M. Collected Papers from the Research Laboratory of Parke, Davis & Co.	437

Kraemer, Henry. Applied and Economic Botany.....	81
Scientific and Applied Pharmacognosy	570
Laubenheimer, K. Allgemeine Bakteriologie und Sterilisationslehre für Ärzte und Pharmazeuten	324
List of Miscellaneous Publications of the United States Public Health Service	530
Mason, Harry B. The Druggist and His Profits.....	436
Window Dressing	573
Merck, E. E. Merck's Annual Report of Recent Advances in Phar- maceutical Chemistry and Therapeutics (1913)	325
Meyer, Arthur. Erstes Mikroskopisches Praktikum. Eine Einfüh- rung in den Gebrauch des Mikroskopes und in die Anatomie der höheren Pflanzen	323
Motter, Murray Galt, and Martin I. Wilbert. Digest of Comments on the Pharmacopœia of the United States of America (Eighth Decennial Revision) and on the National Formulary	86
National Formulary for the Calendar Year Ending December 31, 1913. 86	
New and Non-official Remedies, 1915: Containing Descriptions of the Articles Which Have Been Accepted by the Council on Pharmacy and Chemistry of the American Medical Association Prior to January 1, 1915	291
Pennsylvania Pharmacy Laws, In Effect 1913-1915	438
Pharmacopœia of the United States of America, Digest of Comments on the	530
Reports, Annual, of the Department of Agriculture, 1914, Advance Sheets from	85
of the Surgeon-General of the Public Health Service of the United States for the Fiscal Year 1914	86
Sadtler, Samuel Schmucker. Chemistry of Familiar Things.....	223
Schneider, Albert. Bacteriological Methods in Food and Drug Lab- oratories	573
Sherman, Henry C. Food Products	39
Units of Weight and Measure. Definitions and Tables of Equivalents. 84	
Van Fleet, Walter. Goldenseal Under Cultivation	84
White, E. F. The Spatula Soda Water Guide and for Soda Water Dispensers	326
Wilbert, Martin I., and Murray Galt Motter. Digest of Comments on the Pharmacopœia of the United States of America (Eighth Decennial Revision) and on the National Formulary	86
Year-book of Pharmacy	83
Rice Powder? What is (Lawall)	293
Salol-coating of Pills, Some Experiences with (Peacock).....	514
Salt and Its Relation to Nutrition (Stiles)	220
Sanguinaria, The Proper Time to Collect (Farwell)	97
Science, Progress in Applied	530, 567
Serum, Mercurialized	566
Silver Iodide, So-called Concentrated Solution of (Thum).....	500
Sodium Thiosulphate Volumetric Solution, Standardization of (Mayer).. 154	
Starch, Potato, Selective Stain for	563
Stearic Acid as a Coating for Enteric Pills (Toplis)	518
Sugar Industry, The Development of (Browne)	10
Tablets, Compressed, A Note on (Mueller)	197
Tomato Fruits, The Effect of Certain Conditions Upon the Acidity of (Duggar and Merrill)	277
Toxins, Bacterial <i>Versus</i> Vegetable (White)	127
Tuberculosis, An Experimental Study of a New Remedial Agent and of Its Effects in Pulmonary (Waters)	163
Water, Distilled, Toxicity of (Merrill).....	549



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CONTENTS.

A Comparison of Results Secured by the Use of Chemical and Physiological Assay Methods for Tincture of Aconite. By Charles C. Haskell and H. W. Zirkle, Medical College of Virginia.....	537
A Modification of Rose's Method for the Estimation of Pepsin. By Maurice H. Givens, United States Public Health Service.....	541
Some Relations of Plants to Distilled Water and Certain Dilute Toxic Solutions. By M. C. Merrill, Missouri Botanical Garden.....	549
Concerning Two Sweet-tasting Drugs. By Prof. Rudolph Kobert, Rostock, Germany.....	555
Quarterly Review on the Advances in Materia Medica and Pharmacy. By John K. Thum, Ph.G., Pharmacist at the German Hospital, Philadelphia, Pa.....	559
Progress in Applied Science.....	567
Book Reviews	570
Index to Volume 87 of the American Journal of Pharmacy.....	575

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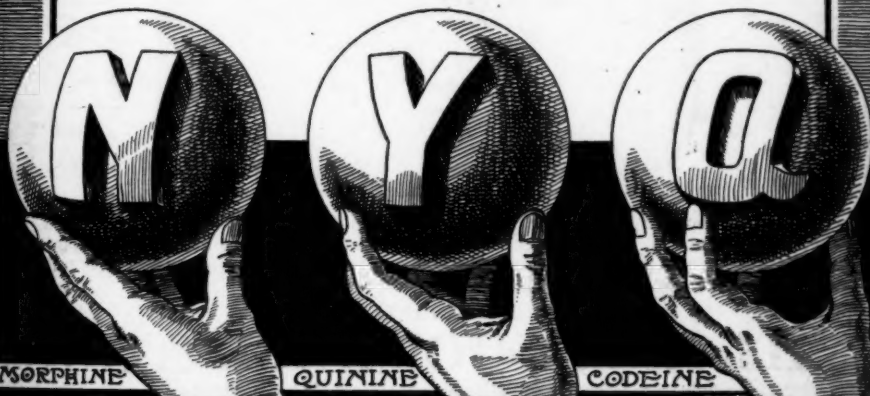
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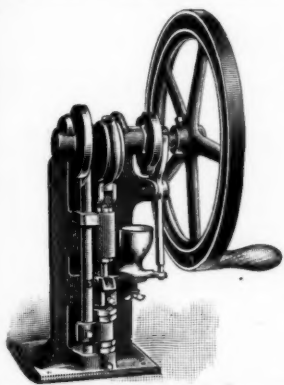
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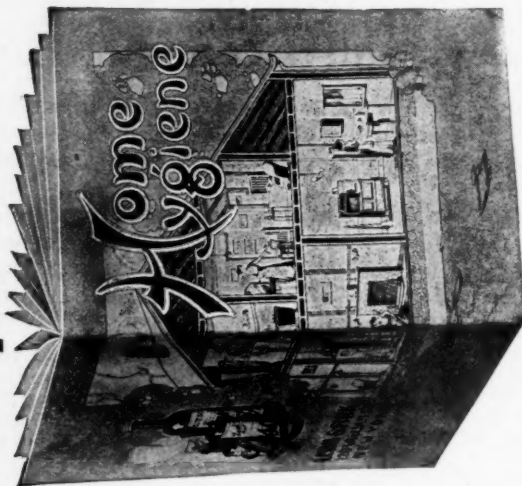
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